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INTERNATIONAL BULLETIN
OF
BACTERIOLOGICAL NOMENCLATURE
AND
TAXONOMY

Volume 9 April 15, 1959 No. 2

INTRODUCTORY REMARKS AND WELCOME*

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Ladies and gentlemen!

To begin with, I wish to express my pleasure that you have so willingly accepted my invitation to discuss here together the methods to be employed for characterising and classifying the streptomycetes. This proves not only that I feel strongly the lack of a usable system for determining these organisms, but that also you who, in most cases, have been dealing with these problems much longer and more in detail, are not satisfied with the schedules existing and described in the literature. What is the reason? Every author has tested with much assiduity and with good observation in most cases thousands of strains and has then tried to establish a system of classification and a key for determination. The situation now is that several keys for determination of the different kinds (species) are available for the genus Streptomyces. Each of these studies in itself is good, and there can be no objection to any of them. However, this perforce results in anybody who deals with streptomycetes—and due to the intensive investigation and research of antibiotica this number is very great nowadays—determining his new isolations by employment of different methods. This procedure is only too conducive to a nomenclature which is the constant source of errors and misunderstandings. At present it is necessary › state in the precise naming of a species what method has been used for testing the particular strain, for often another author classifying a different group may have given the same name to another species. On the other hand, one may conclude

*Presented at Round Table Conference on Streptomyces, August 4-5, 1958, Stockholm, Sweden, 7th. International Congress of Microbiology.

that a new isolate should be named as a new species; however, this isolate when tested according to another method is found to be one that had been described long ago. This procedure has resulted in a legion of new species which certainly can be substantially reduced in numbers by use of the respective comparative tests. Two items could help to overcome these difficulties:

1. Establishment of one system of classification to be used for future work.

2. Creation of Culture Collection for the genus Streptomyces.

For some time the systematic publications referring to streptomyces have been growing. They show, it is true, how much interest is evidenced in the classification and systematics of these organisms, but on the other hand these publications have also increased confusion in this field. You will justly object to my plea for a standardized procedure for testing: Patience, please! The good or the best respectively will make its way alone! This is surely true. But cannot we try to shorten this time of waiting a little by limiting ourselves and by agreeing on the employment of quite definite methods of investigation which we deem necessary and suitable for the description of the streptomycetes?

Permit me for the purpose of comparison, to show you in 2 tables once more the essential points which different authors have taken as bases for their methods of determination, particularly in their more recent studies. Table 1 shows the order of succession in which the different characteristics are used for classification. Krassilnikov, for example, places first the morphology of aerial mycelium, next the shape of the spores when examined under a light microscope, and third the colour of the pigment.

Table 2 is a summary of the statements in Table 1. It shows that the colour and morphology of the aerial mycelium are made use of as the most important characteristics for the primary classification of the streptomycetes. When comparing the different working methods of the authors as given in Table 1, one is struck by the fact that while the three authors Kutzner, Misiek, and Gause (to whom Burkholder with his working proposal may be added as a fourth) proceed by the same path in their determinations; nevertheless the groups, subgroups, and species can be compared only provisionally. The reason is that in all three cases

Table 1. Order of succession of criteria used by different authors for classification

Table 1. Order of succession of criteria used by different authors for classification of the Streptomycetes

Author and year	No. of unit determinations	Color of			Morphology			Physiology		Ecology
						Spore shape				
		Aerial mycelium	Substrate mycelium	Substrate pigment	Aerial mycelium	Light microscope	Electron microscope	Chromogenesis	Antibiosis, etc.	Temperature, etc.
Krassilnikov 1949	53 species	4		3	1	2				5
Waksman and Lechevalier 1953	147 species							3	4	1,2
Baldacci et al. 1954	21 series	2	1						3	
Hesseltine et al. 1954	7 major groups	2			1					
Burkholder et al. 1954		1	2		3				4	
Kutzner 1954,1956	10 major groups 382 subgroups	1	2		4			3	5	
Misiek 1955	9 major groups 31 subgroups 195 color pattern varieties	1	2							
Gause et al. 1957	15 series 108 species	1	2		3					
Pridham et al. 1958	7 sections 42 series	2			1					
Ettlinger et al. 1958	34 species	2			3		1	4		

Literature:

Misiek, M.: Comparative studies of Streptomyces populations in soils, Thesis Syracuse Univ. 1955

Gause, G.F. et al.: Problems of the classification of the antagonistic Actinomyces species, Moskau 1957

Ettlinger, L. et al.: Personal Communication 1958

The remaining literature is given by Baldacci (See pp. 86,87, This BULLETIN)

Table 2. Frequency of the appearance of the most important criteria in the key for identification arranged in the order of their use (Summary of the numbers from Table 1)

	Color of the			Morphology			Physiology		Ecology
					Spore form				
Order	Aerial mycelium	Substrate mycelium	Pigment	Aerial mycelium	Light microscope	Electron microscope	Chromogenesis	Antibiosis, etc.	Temperature, etc.
1	4	1		3		1			1
2	4	4			1				1
3			1	3			2	1	
4	1			1			1	2	
5								1	1

For example: The colour of the aerial mycelium was given first place by 4 authors, by 4 authors second place, and by 1 author fourth place.

different culture media are used in judging the colour of the aerial mycelia, the primary basis of classification. The question whether it is perferable to employ that medium which is optimum for the formation of aerial mycelium in determining its colour, or whether it is better always to use the same culture medium for this reaction, has not yet been decided and will be one objective of our discussion. This illustration indeed broaches the whole question of standardization of the different reactions and culture media. The problem of standardizing the culture substrata, in my opinion, is of decisive importance in order to obtain index numbers and observations which can satisfactorily be compared.

The second item which, by supplementing the first may bring us closer to the ideal, is the establishment of a special Type Culture Collection for Streptomycetes. As to the need for such a standard collection we are agreed—if I understand the letters I have received from you. The manner, and place, and the possibility of financing it must be an important item for consideration.

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Volume 9 No. 2 April 15, 1959 pp. 63-64

UEBER DIE KLASSIFIZIERUNG DER AKTINOMYCETEN ALS PRODUZENTEN VON ANTIBIOTICA*

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1. Eine wichtige Aufgabe auf dem Gebiet der Antibiotica ist die Ausarbeitung von Regeln zur Bestimmung und Unterscheidung der Mikroorganismen, die antibiotische Stoffe produzieren. Die bestehenden Methoden genügen dafür nicht, sie ermöglichen keine Differenzierung der erforschten Organismen. Dieses führt zu grossen Verwirrungen bei der Bestimmung der antibioticaproduzierenden Organismen, wie auch bei der Unterteilung der Antibiotica selbst.

2. Eine der Ursachen der grossen Verwirrung bei der Klassifizierung der Aktinomyceten besteht darin, dass die einzelnen Bearbeiter bei der Erforschung und Kennzeichnung der sichtbaren Merkmale keine einheitliche Methode anwenden. Sie verwenden keine Standardmethoden, weder bei der Zusammensetzung der Nährböden, noch beim Testen der Organismen. Jeder Autor beschreibt die Organismen sehr verschieden, je nach seinem Geschmack und nach seiner Meinung, ohne Rücksicht auf das, was bereits über diese Organismen geschrieben und vorgetragen worden ist. Die Beschreibung eines Stammes durch einen Autor lässt sich oft nicht mit der durch einen anderen Autor vergleichen.

3. Zur Aufstellung einer rationellen Systematik der Aktinomyceten, die Antibiotica erzeugen, ist es erforderlich, einheitliche Grundsätze und Untersuchungsmethoden aufzustellen und eine Beschreibung der Kulturen nach einem einheitlichen Schema durchzuführen, um ihre antibiotischen Eigenschaften nach bestimmten Standarden und Testen festzulegen. Es ist weiterhin dringend erforderlich, dass alle Forscher eine Verabredung treffen, um sich an bestimmte taxonomische Regeln zu halten, die auf dem internationalen Kongress der Mikrobiologen und Botaniker angenommen worden sind.

*Diskussionspunkte für das Streptomycetenmeeting anlässlich des VII. Internationalen Kongresses für Mikrobiologie in Stockholm, August, 1958.

4. Als Grundlage für die Klassifizierung der Aktinomyceten sollte man vor allem die morphologischen Kennzeichen anführen, die Art der sporogenen Hyphen, die Form der Kulturen, den Bau und die Pigmentierung der Kolonien und des Luftmycels. Aber auch die physiologischen und biochemischen Eigenschaften sind wichtig.

5. Zur Erkennung und Differenzierung der Aktinomyceten haben hauptsächlich die spezifischen Wechselwirkungen der Antagonismen eine Bedeutung. Die Erfahrung lehrt uns, dass jeder Art die Synthese besonderer antibiotischer Stoffe eigen ist. Das letztere wird dadurch charakterisiert, dass Stoffe, welche einer gleichen Art entstammen, das Wachstum ihrer Produzenten und Kulturen nicht unterdrücken. Die Kulturen der Aktinomyceten reagieren nicht auf die eigenen Antibiotica. Die Bedeutung dieser Reaktion ist oft begrenzt, sie ermöglicht aber die Auftrennung von Kulturen, welche mit gleichen äusseren morphologischen und kulturellen Anziechen ausgestattet sind.

6. Die als Grundlage zur Spezifizierung geltenden antibiotischen Stoffe kann man voneinander nach den bekannten Beschreibungen in der Literatur unterscheiden. Als Beispiel können wir einige antibiotische Stoffe unterscheiden, die die Aktinomyceten der globisporus-Gruppe bilden. Früher wurde diese Gruppe der Aktinomyceten als einzige Art angesehen, jetzt ist sie in nicht weniger als 8 Arten aufgeteilt worden. Äusserlich sind sie vollig gleiche Typen, in der Bildung von antibiotischen Stoffen unterscheiden sie sich aber scharf voneinander.

7. Durch die Kenntnis der spezifischen antagonistischen Eigenschaften lässt sich die Klassifizierung der Aktinomyceten erleichtern. Sie zeigen sich uns als Hilfsmittel bei der Differenzierung völlig gleichartiger Kulturen der Aktinomyceten. Dabei muss man die sogenannte Erscheinung der "Selbstunterdrückung" berücksichtigen, bei welcher die Kultur das Wachstum der eigenen Zellen unterdrückt. Der Prozess der "Selbstunterdrückung" wird nicht durch Antibiotica hervorgerufen, sondern durch sogenannte "Nekrohormone", in einzelnen Fallen auch durch Phagen. Die ersteren beobachtet man des öfteren bei der Pigmentierung der Aktinomyceten. Die Stoffe, welche eine Lysis hervorrufen und das Wachstum von jungen Zellen der eigenen Kulturen hemmen, sind noch unbekannt. Im zweiten Falle ist die Wachstumsunterdrückung durch besondere Phagen bedingt.

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DIE LOCHKARTEI ALS HILFSMITTEL BEI DER KLASSIFIZIERUNG VON STREPTOMYCETEN

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Wir hielten es für wünschenswert, wenn die allgemeinen Bestrebungen zunächst weniger auf eine strenge Klassifizierung hinzielten, sondern sich zunächst mehr auf eine "vergleichende Systematik" beschränken würden. Noch ist bei den Streptomyceten alles im Fluss und ein eben aufgestellter Bestimmungsschlüssel ist, noch bevor er erscheint, bereits wieder überholt. Bis er jedoch neu aufgelegt wird, vergehen oft Jahre. In der Zwischenzeit aber bestimmt jeder nach dem veralteten Schlüssel weiter, mit dem Ergebnis, dass zahlreiche Streptomyces-Arten in den verschiedensten Laboratorien der Welt "neu" entdeckt werden.

Um dieser Entwicklung entgegenzutreten, schlagen wir die Benutzung einer Lochkartei, anstelle der bisher benutzen Bestimmungsschlüssel, vor. Es wäre sehr zu begrüssen, wenn jeder, der mit Streptomyceten arbeitet, seine "neue" Streptomyces-Art auf eine solche Karteikarte eintragen, und diese zusammen mit dem Stamm an eine noch festzulegende "Internationale Kultursammlung" einreichen würde. Dort würde eine Nachbestimmung durchgeführt, die Karteikarte nochmals überprüft, vervielfältigt, und an alle sich dafür interessierenden Institute und Firmen verschickt. Auf diese Weise würde jeder ständig auf dem Laufenden gehalten werden.

Die Benutzung von Lochkarten würde noch weitere Vorteile bringen. Es wäre so möglich, die enzelnen Arten, hinsichtlich aller Merkmale, viel besser untereinander zu vergleichen, als das beispielsweise bisher der Fall war, oder auf Tabellen möglich ist. Man kann bei der Bestimmung auch weiterhin nach jedem beliebigen Schlüssel vorgehen und so die Zuverlässigkeit der einzelnen Schlüssel prüfen. Werden beispielsweise nacheinander Sporophorentyp, Farbe des Luftmycels, Farbe des vegetativen Mycels, Farbe des löslichen Pigmentes, Braunfärbung auf Peptonnährböden usw., jeweils als Primärmerkmal gewählt, so

muss man, wenn der jeweilige Schlüssel richtig arbeitet, immer bei der selben Art enden.

Auch die Zuverlässigkeit bzw. Variabilität der einzelnen Merkmale kann bei Parallelbestimmungen an den Stämmen der gleichen Art, in verschiedenen Laboratorien, mit Hilfe der Lochkarteikarten sehr leicht ausgewertet werden. Man wird auf diese Weise später einmal exakter entscheiden können, welche Merkmale nun für die Bestimmung von Streptomyceten unbedingt erforderlich und zuverlässig sind, und welche man entbehren kann.

Im dem nachfolgend näher beschriebenen Karteischlüssel haben wir zunächst noch sehr viele Merkmale aufgenommen. Wir sind uns dessen bewusst, dass nicht alle notwendig sind, aber erst die Erfahrung wird zeigen, welche wir vernachlässigen können. Auch die Zahl der benutzten Nährmedien ist noch sehr hoch, und man wird versuchen müssen, diese auf möglichst wenige zu beschränken.

Die Karteikarte* ist nun so angelegt, dass in der linken oberen Ecke die Art- bzw. Stammbezeichnung eingetragen wird. Die 5 oberen Lochpaare (18 - 14) der linken Seite (L) dienen zur Registrierung des Anfangsbuchstabes der Artbezeichnung, z. B. albus = a, wird nach dem Alphabet-Schlüssel gestochen.

Fangen sehr viele Arten mit dem gleichen Buchstaben an, so kann noch der zweite Buchstabe (1, bei albus) mitgestochen werden (Lochpaare 13-9). Auf der rechten Kartenseite wird das von dem Streptomyceten gebildete Antibiotikum vermerkt und mit Hilfe der Lochpaare R 1-5, bzw. 6-10, auf der rechten Seite gestochen.

Auf dem oberen Rand der Lochkarte (O 1-28) werden die morphologischen Eigenschaften und unten (U 1-28) die physiologischen Eigenschaften erfasst. Das Vorhandensein einer bestimmten Eigenschaft wird mit (+), das Fehlen mit (-) bezeichnet. Im ersteren Falle wird tief, im letzteren flach gestochen.

Durch das Lochpaar 28 werden die Stämme mit Luftmycel und Sporenbildung (+) von den unversporten Arten getrennt. Die Lochpaare 24-27 kennzeichnen den Sporophorentyp und die Beschaffenheit des Luftmycels. Für die Charakterisierung der Sporophoren haben wir die von Pridham u. Mitarb.

*Die Karteikarte wurde in Stockholm von Dr. Wallhäusser an die Teilnehmer des Streptomyceten-Meetings verteilt.

(1958) vorgeschlagene Nomenklatur benutzt.

Neben den Sporophorentypen (Kennzahl 1-7) wird in der ersten Rubrik zugleich die morphologische Beschaffenheit des Luftmycels charakterisiert (Kennzahl 8-10). Hierfür haben wir die von Lindenbein vorgeschlagenen Bezeichnungen teilweise übernommen:

pulverulentus	(mehlig, pulverig)
vellereus	(sammetig)
lanatus	(wollig)

Mit Hilfe des Zahlenschlüssels (1, 2, 4, 7-Schlüssel), der hier mehrfach benutzt wird, können bei 4 Lochpaaren 10 Eigenschaften erfasst werden. Die Kennzahlen 1, 2, 4 und 7 sind auf die Schlüsselkarte, oberhalb der Lochpaar-Nummer, aufgedruckt. Die Eigenschaften mit den Kennzahlen 1, 2, 4 und 7 werden gemäss dem Schlüssel tief, die restlichen flach gestochen.

Sporen-Form (1, 2, 4, 7-Schlüssel)

Zur Charakterisierung der Sporenform werden lichtmikroskopische und elektronenmikroskopische Beobachtungen herangezogen. Als Standardnährboden wird Stärkeagar benutzt. Die Kennzahlen 1-3 beziehen sich auf die lichtmikroskopisch erfassbaren morphologischen Eigenschaften [(sphäroid (kugelig), langoval und zylindrisch)] und die Kennzahlen 6-9 auf die Charakterisierung der elektronenoptisch beobachteten Anhänge, bzw. Oberflächenbeschaffenheit der Sporen.

Farbe von Sporen und Luftmycel (1, 2, 4, 7-Schlüssel)

Die Angabe der Farbe erfolgt nach den auf Glukoseagar bzw. Emerson-Agar gemachten Beobachtungen. Es wurde dabei bewusst auf die Angabe von Zwischentönungen (z. B. hellgrau, mausgrau, usw.) verzichtet, und nur 10 Hauptfarben festgelegt.

Vegetatives Mycel (Substrat-Mycel)

Die Angaben erfolgen nach den Ergebnissen auf Glukose-Agar, Emerson-Agar und Stärke-Agar. Zur Beurteilung der Beschaffenheit des Mycels werden 8 Eigenschaften, zur Charakterisierung der Färbung 10 Hauptfarben aufgeführt.

Lösliches Pigment

Unter "löslichem Pigment" verstehen wir gefärbte Substanzen, die in das Nährmedium (Agar oder Kulturlösung) ausgeschieden werden und dieses verfärben. Die pH-Abhängigkeit der Farbe wird durch Extraktion der Kulturlösung mit n-Butanol bei pH 2 und 8, und des Mycels mit Methanol, geprüft. Nachdem Eindampfen des Butanols bzw. Methanols im Vakuum erfolgt die Prüfung auf Wasserlöslichkeit. Butanolische, methanolische und wässerige Lösung werden anschliessend unter der UV-Lampe auf das Vorhandensein fluoreszierender Eigenschaften untersucht.

Für die Einstufung in die Farbskala wurden 7 Grundfarben gewählt.

Auf der unteren Seite der Schlüsselkarte sind die physiologischen Eigenschaften aufgeführt.

Zur Abtrennung jener Arten, die auf Peptonnährböden (Pepton-Gelatine) eine Braunfärbung ergeben, dient das Lochpaar Nummer 1.

Für die Bestimmung des Temperaturoptimums haben wir lediglich zwei Temperaturstufen gewählt, 22 und 37°C. Gerade hierbei ergeben sich, hinsichtlich der enzymatischen Reaktionen, markante Unterschiede.

Produktion antibiotisch wirksamer Substanzen

Wir haben uns auf einige wenige Aussagen beschränkt, die zunächst nur orientierenden Charakter haben und die mit verhältnismässig einfachen Versuchen ermittelt werden können. So wird erfasst, ob das Antibiotikum in der Kulturlösung oder im Mycel vorkommt und ob es daraus mit Butanol (pH 2 und 8) bzw. Methanol extrahierbar ist. Durch Verwendung von Testorganismen, die gegen bekannte Antibiotika resistent gemacht wurden, lässt sich auch die Gruppenzugehörigkeit bestimmen. Weiters Aussagen, welcher Vertreter dieser Gruppe nun vorliegt, lassen sich jedoch nur an Hand papierchromatographischer Untersuchungen machen.

Die Festlegung des orientierenden Wirkungsspektrums (Kennzahlen 1-4) erfolgt im Strichtest. Die Auswertung gegen Trichomonaden, Viren und Sarkome—die Aktivität geht hier Häufig mit der Wirksamkeit gegen Hefen parallel—dürft wohl von besonderem Interesse sein, wird aber wohl nicht in jedem Laboratorium durchgeführt werden können.

C- und N-Quellen-Spektrum

Die Versuche zur C-Quellen- und N-Quellen-Verwertung führen wir in graduierten 10-ml-Zentrifugenglassern, mit 3 ml Füllung, durch. Die Auswertung erfolgt nach 5-tägiger Kultivierung auf der Schüttelmaschine (200 Umdr. /Min.) bei 28°C, durch Zentrifugieren, 10 Min. bei 6000 Umdr. Der prozentuale Anteil des Sedimentes wird bestimmt und mit der Normalkultur (Czapek-Dox-Lösung) verglichen. Auch hier lässt sich sicherlich die Zahl der verwendeten C-Quellen noch einschränken.

Eine ausführliche Arbeit zu diesem Thema erscheint demnächst im "Archiv für Mikrobiologie."

INTERNATIONAL BULLETIN

Streptomyces	gebildete Antibiotica:
Sporophoren-Typ:	Aktivität in:
Sporen-Form:	Kulturlösung: extrahierbar:
Sporen-Farbe:	Mycel: extrahierbar:
Farbe des Luftmycels:	**Wirkungsspektrum:**
Farbe des vegetativen Mycels:	Staph. aureus
Lösliches Pigment:	Staph. tetracyclin resist.
Farbe pH abhängig:	Staph. macroliden resist.
Braunfärb. auf Pepton Nährb.:	Staph. albamycin resist.
Stärkehydrolyse:	
Gelatineabbau (bei 18° C):	E. coli
Caseinhydrolyse:	Ps. aeruginosa
Milchkoagulation:	Mycobakterien
Milchpeptonisierung:	C. albicans
Ca-Malat-Abbau:	Asp. niger
Cellulose-Abbau:	
Nitratreduktion:	**Literatur:**
Temperatur-Optimum:	

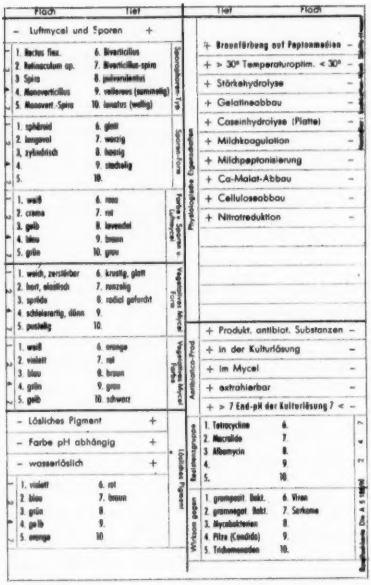
Bandlochkarte Din A 5 160/H

Lochkarte Nr. 1

Nährboden	Wachstum	Reform Oberfläche, Rand, Myzel	Luftmycel u. Sporen-Farbe	Lösliches Pigment	Bemerkungen
Glukose-Asparagin-Agar					
Glukose-Agar					
Nähr-Agar					Hydrolyse: optimal bei °C
Stärke-Agar					
Synthet.-(Czapek-Dox)					
Emerson-Agar					Abbau:
Ca-Malat-Agar					
Casein-Agar					Hydrolyse: optimal bei °C
Cellulose-Agar					Abbau:
Kartoffel-Agar					
Nähr-Gelatine					
Nitrit-Medium					Nitratreduktion:
Lackmus-Milch					Koagulation: Peptonisierung:
Kartoffel-Scheibe					
C-Quellen Verwertung	L-Alkan L-Arabinose L-Rhamnose	D-Fructose D-Galaktose D-Glucose	Maltose Lactose Raffinose	Inulin D-Mannit D-Sorbit	Diulcit Mesoinosit Salicin
N-Quellen Verwertung	Nitrat: NH_4	Asparagin Pepton Lysin			

Lochkarte Nr. 2

BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY



Lochkarte Nr. 3

INTERNATIONAL BULLETIN OF BACTERIOLOGICAL NOMENCLATURE AND TAXONOMY
Volume 9 No. 2 April 15, 1959 pp. 73-78

FACTORS TO BE CONSIDERED IN THE CLASSIFICATION OF ACTINOMYCETES*

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The important factors to be considered in regard to the classification of actinomycetes pertain primarily to the recognition of genera and species. Group characteristics are only of secondary importance.

Recognition of genera

All the accumulated evidence points to the recognition, at the present time, of 10 genera in three families of the class Actinomycetales, namely, the Actinomycetaceae, the Streptomycetaceae, and the Actinoplanaceae. These can be classified as follows:

A. Spores formed, but not in sporangia.
  I. Substrate mycelium fragmenting into bacillary or coccoid elements.
    Family I. Actinomycetaceae Buchanan
    1. Anaerobic or microaerophilic, parasitic, not acid-fast. 1. Actinomyces Harz.
    2. Aerobic, some parasitic, partially acid-fast or nonacid-fast. 2. Nocardia Trevisan.
  II. Substrate mycelium nonseptate, not fragmenting into bacillary or coccoid elements.
    Family II. Streptomycetaceae Waksman and Henrici
    1. Aerial mycelium not produced.
      a) Spores formed singly on short sporophores.
        a_1. Mesophilic forms.
          3. Micromonospora Ørskov
        b_1. Thermophilic forms.
          4. Thermomonospora Henssen

* Presented at Round Table Conference on Streptomyces, August 4-5, 1958, Stockholm, Sweden, 7th. International Congress of Microbiology

2. Aerial mycelium produced.
   a) Spores formed in chains; mesophilic and thermophilic forms.
      5. Streptomyces Waksman and Henrici
   b) Spores formed singly; thermophilic forms.
      6. Thermoactinomyces Tsiklinsky
   c) Spores formed in pairs.
      a_1. Mesophilic forms.
         7. Waksmania Lechevalier and Lechevalier (Microbispora Nonomura and Ohara)
      b_1. Thermophilic forms.
         8. Thermopolyspora Henssen

B. Spores formed in sporangia.
   Family III. Actinoplanaceae Couch
   I. Aerial mycelium usually not formed, coiled conidiophores lacking, sporangiospores motile.
      9. Actinoplanes Couch
   II. Aerial mycelium abundant; coiled conidiophores as well as sporangia formed in some species, sporangiospores nonmotile.
      10. Streptosporangium Couch

Recognition of species

The problems involved in species differentiation of actinomycetes, especially within the genus Streptomyces, must take into consideration primarily morphological and cultural criteria. Some of the biochemical characteristics offer supplementary information. These properties may be briefly summarized as follows.

1.) Morphology includes the structure of the colony and of the substrate mycelium; the nature of the aerial or surface mycelium and manner of sporulation; the size, shape, and surface structure of spores.

2.) Cultural properties include growth characteristics on certain organic and synthetic media. Solid media are primarily used. The common liquid media do not offer much supplementary information. Pigmentation of the aerial and substrate or vegetative mycelium, formation of soluble pigments, proteolytic activities, notably gelatin liquefaction and milk coagulation and proteolysis, as well as growth

on potato and on certain other selected media, are most important.

3.) The biochemical activities include, in addition to the above, sucrose inversion, diastatic and reducing properties, utilization of different carbon sources, and formation of antibiotics.

4.) Sensitivity to specific phages, and, to some extent, serological reactions, offer additional specific characteristics.

5.) Standard media must be adopted for purposes of characterization. These comprise both well-defined solid synthetic media and carefully selected organic solid and liquid media. Conditions of growth for the purpose of species characterization should be constant.

6.) Certain important characteristics should also be recognized for establishing strains or varieties of organisms within a single species. These are usually based upon morphological and cultural variations, upon the ability to produce economically important substances, such as vitamins, enzymes, and antibiotics, and upon other properties, such as cross resistance, phage and antibiotic sensitivity.

7.) Type cultures should be properly preserved, deposited in recognized culture collections, and made available to all investigators throughout the world.

8.) No scientific journals should accept contributions in which a new species is listed without a proper description of the organism in a recognized international language or a reference to a previously published description, and without its prior deposition in a recognized culture collection.

Recognition of groups

It has been recognized, since the early days of the study of actinomycetes, that some of the forms, now found to belong largely to the genus Streptomyces, represent groups of organisms rather than single species. Sanfelice, as far back as 1904, suggested the division of the genus, designated as Streptothrix, into three groups: S. alba, S. flava, and S. violacea; it is quite evident from these designations that pigmentation was used as the basis for group separation. Waksman (1919) also recognized the need for separating the aerial mycelium-producing actinomycetes into species-groups, on the basis of their morphological and cultural properties. He demonstrated three morphological types,

those forming straight sporulating hyphae, spiral-shaped hyphae, and verticil-forming organisms. He stated "often several strains of the same 'species-group', when compared on the same culture medium might be taken culturally and often morphologically as distinctly different species."

More recently, Baldacci et al. (1954) suggested a division of the organisms belonging to the genus Streptomyces into "sections" and "series," largely on the basis of color of the substrate and of the aerial mycelium, respectively. Each "series" was to be divided into species on the basis of enzymatic activity, antibiotic properties, and soluble pigments. Twenty-one series were thus recognized. This grouping was later variously modified by Baldacci (1956).

Gause et al. (1957) presented a rather elaborate system of classification of the genus Streptomyces using a set of rather peculiar assumptions. On the one hand, the generic name Streptomyces was not recognized officially; on the other hand, however, reference to all other species, usually included under the genera Actinomyces, Micromonospora, and others, was excluded, thus automatically recognizing the genus Streptomyces by an omission which consisted in carefully avoiding this name.

The separation of the genera into groups is based, first of all, on the pigmentation of the substrate growth and of the aerial mycelium; some of the morphological properties of the organisms, notably manner of sporulation and nature of spores, are also taken advantage of in the separation of the genus Streptomyces into groups. It must, of course, be kept in mind that any attempt to separate the genus Streptomyces into groups will accomplish no more than the raising of some of the important species to subgeneric status.

Variability

Any system of classification of such a variable group of microorganisms as the streptomycetes must recognize the continuity between different species. Allowance must also be made for transitional forms which would tend to bridge the gaps between the individual species. The need for a natural system of classification is as great for the Actinomycetales as for any other group of bacteria. One must consider, first of all, the morphological properties, especially the formation of aerial mycelium and the type of sporulation. Then come the cultural properties, notably

pigment formation and growth upon different media. Consideration must also be given to the ecological properties, notably anaerobism vs. aerobism, pathogenicity vs. saprophytism and thermophily vs. mesophily. There are, further, the physiological activities, including carbon and nitrogen utilization. Finally, there are the chemical properties (composition of cells) and the biochemical activities, such as the property of formation of antibiotics, enzymes, and vitamins. All of these can serve as supplementary characteristics for describing species and varieties of actinomycetes.

Recommendations

It is proposed to utilize a combination of ecological, morphological, and certain physiological properties of the organisms for the characterization of genera of actinomycetes. Some of the morphological, cultural, and physiological properties may be used in establishing groups within the genera. Cultural and biochemical properties are utilized for characterizing species. Finally, the recognition of varieties must be left to the individual investigators, who may consider such problems as variability and specific biochemical activities, such as sugar utilization and antibiotic production.

Formation of antibiotics should certainly be considered among the properties for species characterization. Carbon sources as a specific property is open to question; it may be left to varietal characteristics.

ADDENDUM

Proper use of the vernacular words "actinomyces" and "actinomycetes", "streptomyces" and "streptomycetes"

Ever since Harz gave the first valid description of an actinomycete in 1877, there has been considerable confusion as to the nomenclature of this group of organisms. There are certain reasons for this confusion: 1. The close similarity between the scientific name Actinomyces and the vernacular names "actinomyces" and "actinomycete" has tended to confuse the workers in this field. 2. Especially since 1943, when Waksman and Henrici proposed the new genus

Streptomyces, which included many, but by no means all, of the species previously assigned to the genus Actinomyces, the situation has tended to be further confounded.

Recent literature on the actinomycetes is replete with many examples of the use of the words "actinomyces" and "Actinomyces" where it is virtually impossible to determine whether the author is referring to all of the actinomycetes, to the genus Streptomyces, or to the genus Actinomyces. In fact, the writer readily admits to such confusing terminology in some of his own publications. To avoid at least some of this difficulty, the writer makes the following proposals:

1. The name "actinomyces" may be used as the vernacular expression only for members of the genus Actinomyces; it should no longer be considered as synonymous with the singular "actinomycete" or the plural term "actinomycetes." Likewise "streptomyces" is the acceptable vernacular name when used to refer solely to members of the genus Streptomyces.

2. The vernacular terms "actinomycete" and "actinomycetes" can properly be used as inclusive terms for any or all of the organisms now included in the Actinomycetales, exclusive of the mycobacteria and corynebacteria, without referring to either families or genera. The words "streptomycete" and "streptomycetes" should be limited to members of the family Streptomycetaceae.

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BERICHT UEBER UNTERSUCHUNGEN ZUR SYSTEMATIK DER STREPTOMYCETEN*

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Im Rahmen einer Arbeit über die Ökologie der Streptomyceten wurden mehrere hundert Stämme isoliert. Da sich eine Identifizierung des ganzen Materials nach den zur Verfügung stehenden Bestimmungsschlüsseln als zu schwierig erwies, wurden lediglich alle 1856 Streptomyces-Stämme in einer einheitlichen Weise durch folgende Merkmale charakterisiert: Erscheinungsform auf mehreren Nährböden und Nährlösungen, Morphologie des Luftmycels und der Sporen, Bildung eines melanoiden Pigmentes auf peptonhaltigen Nährböden, antibiotisches Spektrum (5 Test-organismen). Die Merkmale wurden in übersichtlicher Weise auf Karteikarten eingetragen, so dass schnell und einfach ein Vergleich verschiedener Stämme vorgenommen werden konnte. Die Schwierigkeiten, die bei der Beschreibung bzw. Benennung der Farbtöne bestehen, wurden dadurch umgangen, dass die Beurteilung nach der Farbtafel nach OSTWALD vorgenommen wurde.

Die Farbe des Luftmycels erwies sich als so charakteristisch, dass sie zur Einteilung der Stämme in Hauptgruppen herangezogen wurde. Die zahlreichen grausporigen Stämme wurden nach der Farbe des Substratmycels weiter unterteilt. Innerhalb der auf diese Weise gebildeten 10 Hauptgruppen wurden die sich in den festgestellten Merkmalen als gleich oder sehr ähnlich verhaltenden Stämme zu Untergruppen--insgesamt 382--zusammengestellt. Die zunächst hoch erscheinende Zahl verschiedener Untergruppen rührt daher, dass oft schon geringfügige Unterschiede berücksichtigt wurden. So enthalten 42% der Untergruppen nur einen Stamm und 18% nur zwei. Jedoch gibt es auch zahlreiche Untergruppen mit mehreren und zudem aus jeweils

*Diskussionspunkte für das Streptomycetenmeeting anlässlich des VII. Internationalen Kongresses für Mikrobiologie in Stockholm, August, 1959.

verschiedenen Bodenproben isolierten Stämmen, zB. 18 mit 5 Stämmen, 8 mit 10, 9 mit 11, 3 mit 27.

Für jede Hauptgruppe wurde ein dichotomer Bestimmungsschlüssel aufgestellt, wobei als erstes Differenzierungsmerkmal die Pigmentbildung auf Peptonnährboden, die Morphologie des Luftmycels und die antibiotische Aktivität verwendet wurden.

Die Beschreibung der einzelnen Untergruppen wurde in Tabellenform vorgenommen, d. h. die einzelnen Merkmale einer Untergruppe wurden nebeneinander aufgeführt, so dass Unterschiede zwischen "benachbarten" Untergruppen sehr leicht zu übersehen sind, zumal die Farbmerkmale durch kleine aufgeklebte Farbscheibchen angegeben werden. Diese Anordnung der Merkmale bei Stamm oder Artbeschreibung wird wegen ihrer Übersichtlichkeit einer weiteren Anwendung empfohlen.

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CRITERIA FOR THE IMPROVEMENT OF THE CLASSIFICATION OF ACTINOMYCETES*

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1. Characteristics and Reactions Useful in Classification

The elements accumulated during the last few years which may be most useful for the improvement of the classification of Actinomycetes, are in our opinion the following:

a. Knowledge of the microscopic structure and particularly the structure of sporophores. Contributions in this field have been made by Krassilnikov (13), by Burkholder and coll. (7), by Hesseltine and coll. (11) and by Pridham and coll. (18).

b. A knowledge of the shapes of the spores and their appendages under the electron microscope. In this connection we note the works of Kriss (14), Küster (10, 15) and those of Baldacci and coll. (4, 5, 6).

c. The choice of those morphological characters which are less subject to variation or do not vary at all in relation to the use of various cultural substrates. Contributions in this field have been made by Baldacci and coll. (2, 3), by Flaig and Kutzner (9), by Kutzner(16), and by Hesseltine, Benedict, Pridham (11).

d. Production of catabolic products and in particular antibiotics. Contributions in this field are too numerous to be mentioned (Waksman 19, 20).

The problem lies in the method of utilization and the setting in order of these elements for the purpose of a rearrangement of the classification of Actinomycetes.

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Solutions of this problem have been suggested by single investigators, but these solutions are generally based on results of their own work. Thus several research workers have created new genera such as: Microbispora by Nonomura and Ohara (17), Streptosporangium and Actinoplanes by Couch (8), Thermomonospora, Thermopolyspora, Thermoactinomycetes, Pseudonocardia by Henssen (12). Others have preferred to form series or groups or sections rather than genera, as Baldacci and coll. (2, 3), Flaig and Kutzner (9), Pridham and coll. (18).

On the other hand the excessive multiplication of species has been principally the work of those who have devoted themselves to the examination of catabolic products such as pigments and above all antibiotics (Baldacci, 1).

A brief summing up of the situation shows the absence of a clear and systematic method of classification for this group of microorganisms. And to this must be added the fact that according to some investigators Actinomycetes should be considered as bacteria whilst according to others they are fungi. No definite view can yet be expressed on this matter as there are no adequate reasons in support of either one or the other of these theories.

2. Choice of Media and Their Standardization

The choice of media for classification purposes must satisfy two requirements:

A. In the first place media must be prepared which are suitable for comparison with the diagnosis made by earlier research workers, that is to say we must in each case use media that have already been used by our predecessors. This consideration is put forward out of respect for the researches made by others and also because it is expedient to utilize to the utmost all preceeding nomenclature. We know with absolute precision, because we have taken the trouble to read

it up, that for example, the diagnoses of Actinomyces albus, Actinomyces sulphureus, Actinomyces Bostroemi, Actinomyces Madurae, made by the respective European investigators who were the first to study these Actinomycetes, leaves no doubt about the possibility of their re-identification. We regret that this criterion, which we have faithfully followed has not been duly appreciated by English-speaking research workers, and that these species are generally almost completely ignored by them.

B. The standardization of these substrates which may be effected today thanks to the supply of all sorts of chemical substances, must be the result of work done at the "round table". The choice of substrates must be subject to the condition of the characters that are to be utilized for the classification key.

3. The Choice and Hierarchy of Characters for Application in a Classification Key

Among the reasons put forward to explain the difficulty of defining the species among the Actinomycetes is the necessity of introducing certain characters not used by botanists. The introduction of new characters in a classification should be made with due respect for the definition of the concept of the species which is nothing more nor less than a concept of grouping. Every time a scientist indicates several objects with the same name, he asserts not only that they resemble each other but also that they resemble each other in some thing; that is to say that there are determining characteristics common to all which can be enumerated and designated separately.

It is evident that the choice of new characters to be utilized presupposes that they are definable and countable, and they should be easily recognizable so that there may be no difficulty in their recognition and designation and no fear of errors.

They should be constant, that is to say independent at the highest possible level of external or foreign factors, or at least they should remain constant under conditions easily reproducible by every investigator.

I do not know how many so called biochemical character-

istics used in the classification of the Actinomycetes have been formed by taking into account these requirements. It seems rather that they are the work of specialists in other branches of science rather than that of systematic workers. It would seem legitimate to ask oneself if the failure of classification be not rather the consequence of an erroneous choice.

The choice of characters presupposes not only recognizability and countability but also a criterion of their hierarchy, because this is a specific requirement of any classification system. That is to say the choice of characters presupposes the possibility of giving them a suitable hierarchical position, at the various levels of grouping. For this reason the formation of genera based upon obsolete characters, for example, the creation of the genera of Actinomycetes for their resistance to a high temperature, seems to be arbitrary.

It is easy to see than an important pre-existing attribute does not exist a priori in the characters to be utilized.

The choice of hierarchy or succession obeys another general principle which is the foundation of every scientific system whether it be explanatory or classifying. That is the intrinsic possibility of the development of the system. As Planck said in a celebrated passage "As long as man persisted in dividing all natural forces into friendly or hostile forms, plants into poisonous and edible, animals into dangerous and harmless, he never attained true science."

Have not many microbiologists perhaps fallen into the same error by claiming that the Actinomycetes should be grouped together as pathogenic and nonpathogenic (for man), as useful (because they produce antibiotics) or useless (because they don't)?

In scientific language, and implicitly in a system of classification, every character should be chosen and defined in an univocal manner; but this choice and this definition must not have reference to anything that is outside the system that it is desired to construct, as for example, its advantageousness or disadvantageousness for man, but to something that is in the system itself, in the intrinsic reasons of the system. One must be careful to arrange the succession of characters, which have been collected and defined one by one, in such a way as to facilitate the work of distinguishing and forming the species.

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On the basis of what we have set out also in paragraph 1, we propose:

a. Confirmation and new formation of genera utilizing the characteristics of the microscopic structures of the sporophores.

The following genera are cited as examples:

Micromonospora
Microbispora
Streptosporangium
Actinoplanes
Streptomyces (or Actinomyces)

For the Actinomycetes with verticillate sporophores, although only a small number has been studied, we propose the genus Streptoverticillium [genus that groups together the sections Monoverticillus, Monoverticillus-spira, Biverticillus, Biverticillus-spira of Pridham and coll. (18)].

b. Confirmation and new formation of species utilizing the morphological characters of the vegetative and aerial mycelium and of the shape of the spores under the electron microscope. These species should replace the present series or sections or groups. This proposal modifies our previous scheme. (Baldacci and coll. 2, 3).

c. Grouping on the level of varieties or clones the descriptions of species that utilize the characters that are biochemical, ecological, producing antibiotics, etc.

The nomenclature of the genera, species and varieties should be a work of revision at the "round table" and should follow the rules of The International Code of Nomenclature of Bacteria and Viruses and the International Rules of Botanical Nomenclature.

In particular we wish to draw attention to our previous discussion of the subject [Baldacci and coll. (2)].

4. Conditions of Conservation

Observations on this matter have been set out in our work with collaborators.

5. Creation of a Type Culture Collection

We are in favour of this once the conditions of culture and conservation referred to above have been agreed to.

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SYSTEMATIC VS. CLASSIFICATORY TAXONOMY AND SPECIOLOGY OF THE ACTINOMYCETALES*

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Recent development of theoretical taxonomy, at least in Italy, demands that the taxonomy of microörganisms be distinguished as systematics and classification.

Systematic taxonomy (or systematics) distributes the microörganisms of a certain rank with due consideration of all the affinities, immediate or remote, and the probable descent. Their interrelationships may be expressed by development of a phylogenetic tree. This is, indeed, as far as it is realizable, a "natural" taxonomy, developing an increasing complexity with the progress of knowledge, but more or less objective.

Classificatory taxonomy (or classification) distributes the microörganisms of a certain rank on the basis of a reduced (small) number of easily recognizable characteristics showing divergences or similarities, without consideration of relationship or descent. The affinities or divergences are expressed in keys (frequently dichotomous). This is, of course, an artificial taxonomy, simplified and more or less subjective. (It is always possible to develop analytical keys from systematics in order to ease the routine work of determination).

Most taxonomy is mixed, but the systematic stage is increasingly evident with the advance of our understanding of each group of microörganisms.

This twofold scheme is obvious in any arrangement. A book of unknown content may be "classified" according to a number of physical characteristics such as weight, size, number of pages, paper color or quality, etc., but it may be "systematized" only after knowledge of the content. A better knowledge of the content will correspond to a better systematization of the book (e.g. according to a decimal or similar library classification).

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The taxonomy of the Actinomycetales is still in the classificatory stage. Specialists may indeed agree to select certain characteristics and to establish their hierarchy in order to develop a standardized classification in agreement with actual knowledge.

The taxonomy of microörganisms is complicated by the formal levelling of "species", delimited on the basis of deeply divergent criteria. In general we may distinguish:

A) Morphologic criteria
   a) Macromorphologic characteristics (in one case of the Actinomycetales, used more frequenlty for genera than for species).
   b) Micromorphologic characteristics (seldom used).
   c) Biometric characteristics (of unusual application).

B) Biologic criteria
   a) "In vivo" specialization.
      1) Host specialization (used for Actinomycetales parasitic on man, animals, or plants).
      2) Organotropic specialization (as above).
      3) Pathognomic characteristics (as above).
   b) "In vitro" specialization.
      1) Physiognomic characteristics (e.g. color and shape of colonies on solid and liquid media) (of common use).
      2) Physico-chemical characteristics (e.g. growth response to temperature; liquefaction of proteins; milk peptonization) (of common use).
      3) Biochemical characteristics (e.g. H_2S production; NO_3 reduction; C and N compound utilization; antibiotic production; sensibility to antibiotics; nature of pigments and cell-wall; serological reactions) (of common use).

The following rules are proposed:

1. Acceptance of specific rank with Latin binomial for the 'series', namely, complex of clonal, applied populations (as, e.g., in several genera of molds). Infraserial clones (strains) are designated with the binomial of the series followed by numbers or letters;
2. Species ('series') are accepted (recognized) only if

they differ in at least two characteristics not included in the same group of criteria, e.g. not only biochemical or cultural, etc. (Apparently speciation in nature is achieved by several, parallel and simultaneous modifications);

3. Strains differing for one or a few allied characteristics are infraspecific (infraserial) (unnamed) taxa;

4. Description and recognition of species ('series') and of individual clones must be checked by qualified specialists, with sufficient knowledge of the group and in possession of cultures suitable for comparison;

5. Cultures should be distributed among specialists and Type Culture Collections.

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CONTRIBUTION TO AN ESSAY ON THE MORE RATIONAL CLASSIFICATION OF ACTINOMYCETES*

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1. Present chaos in the classification of the actinomycetes derives to a great extent from the concept of a majority of microbiologists regarding species in general and especially with respect to microorganisms. The point of view should be finally abandoned that species are not realities in nature, that is, that they are solely taxonomic conceptions. Such ideas have led to the splitting of species on the basis of a few and unstable characteristics and to the formation of "splinter, artificial species" which actually do not exist in nature. Therefore, we should start from the principle that species are realities in nature, that they are representatives of a definite stage in the evolution of living things under determined ecological conditions, that they represent a group of generations derived from a common ancestor. These generations in the course of their struggle for existence and through natural selection have been distinguished from other representatives by a complex of different characteristics (morphological, physiological, biochemical, serological). In reality we are concerned with larger "lumped natural species" which comprise a series of varieties, biotypes, and strains. This concept in fact gives a picture of the real state in nature, since these forms actually delimit the species in nature.

2. It is evident that the immense amount of reference material, collected during the past decades of studies on actinomycetes, cannot be filed and classified within the present narrow framework now used for these microorganisms. Because of this increasing mass of material there is need to widen the present scope and format. Therefore, we propose that classification units should be extended below and

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above the present species level in accordance with the International Code of Microbiological Nomenclature. Certain transitory systematic categories should be introduced also, if such are found to be necessary. In this way it would be possible to arrive at "more natural" systems of biological classification of these microorganisms based on thorough and extensive knowledge of their properties.

3. It is necessary to introduce a definite hierarchy of characteristics on the basis of which the classification of the actinomycetes and other microorganisms may be elaborated. These criteria should be based exclusively on their constancy: the more constant properties should constitute the basis for higher categories of taxa, and the less constant for the lower categories. It is believed to be evident that under natural living conditions of microorganisms and on the basis of changes in the ecological factors of the surroundings, there occur certain less significant changes of the ferment systems and biochemical properties of microorganisms. These changes are, however, reflected in changed physiological properties, so that their morphological properties finally become manifest. We should, therefore, consider the morphological characteristics as the most constant and take them as a basis for higher systematic categories. Morphological characters would be followed by macroscopic physiological characteristics in nature, and these by the biochemical characteristics as most subject to change. On these latter the infraspecific systematic categories should be based.

4. We are proposing, therefore, that the classification of the actinomycetes should start with the determination of their morphological properties (microscopic structure of the mycelium, sporophores, spores) thus enabling one to determine certain higher systematic categories. One can then proceed with the determination of the physiological properties (macroscopic aspect of the cultures, pigmentation), and finally deal with the biochemical properties (production of antibiotics, vitamins, etc.) upon which the grouping of varieties, biotypes and strains of the particular larger "natural" species should be based. The relationship between the microorganisms and the ecological factors (temperature, oxygen, hosts, etc.) should not be taken as a basis for their classification (especially with regard to the higher systematic categories), since this relationship is

in general already reflected in the properties noted above. Not even the economic significance of each strain of these microorganisms should have any influence on the nature of their biological classification.

5. Finally, we completely agree that the creation of a collection of type cultures should be made possible for these microorganisms also. We should then proceed with the selection and establishment of the standard culture media (mostly synthetic in nature) and of the conditions for their laboratory studies. The electron microscope, although giving reliable information, is not always accessible to many microbiological laboratories of the world, and it is too early to base the classification of the actinomycetes on data derived from them. Ultimately, our aim should be the realization of a simpler system of classification of these microorganisms. Should we take some of the above mentioned principles as a basis, we believe that even then we should not have to deal with such a great number of the larger natural species of actinomycetes as some microbiologists have envisioned. However, the number of varieties, biotypes and strains, particularly those of great economic significance would, no doubt, be increased, but this is quite justifiable. In view of this fact, we are submitting a simplified schema of such a system of classification of these microorganisms for discussion.

Class I. -ACTINOMYCETALIA-(Actinomycetes) Mycelium absent or present
Subclass I. -ACTINOMYCETINEAE - Mycelium present in early stage of cultures

Order I. -Proactinomycetales - Mycelium septate
Family I. - Proactinomycetaceae - "Spores" in chains
Genus I. - Proactinomyces - Aerial hyphae ("sporophores") absent
Genus II. - Nocardia - Aerial hyphae ("sporophores") present

Order II. -Actinomycetales - Mycelium nonseptate
Family I. - Micromonosporaceae - Spores single
Genus I. - Micromonospora - Sporophores short

Family II. - Actinomycetaceae - Spores in chains
Genus I. - Actinomyces - Sporophores straight
Genus II. - Streptomyces - Sporophores spiral

Subclass II. -MYCOBACTERIINEAE - Mycelium absent in early stage of cultures

In summary, a class is defined by the presence or absence of mycelium; subclasses, however, by the presence of mycelium in early stages of cultures; orders by septate or nonseptate mycelium; families by the groupings of the spores; genera by the presence and shape of sporophores, while the determination of the species, varieties, biotypes and particular strains is based on the shapes and structure of the spores themselves, the colour of the aerial and substrate mycelium and the production of pigments, as well as the other physiological and biochemical characteristics (sources of carbon, of nitrogen, of ferment systems; production of antibiotics and biotics, etc.).

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OUTLINE OF A COMPARATIVE STUDY OF CRITERIA USED IN CHARACTERIZATION OF THE ACTINOMYCETES

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Taxonomy of the Actinomycetales

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In the course of the meeting on Streptomycetes in Stockholm, August, 1959, the participants formulated a plan for a common experiment which should establish the reproductivity of the stable properties used in the characterization of streptomycetes. In the various laboratories, the same strains on standardized culture media are to be examined and judged at determined intervals for the expression of certain properties. The following outline of the plan was distributed to those who agreed to participate in the experiment. Thirty four individuals and laboratories agreed to participate.

Outline of Experimental Plan

General Experimental Conditions:
Altogether, the experiments will be carried out three times in a dark room with a temperature of 28° C. The interval between repetitions is 2 months, during which the strains will be reinoculated three times. For a growing period of 12 days the cultures are stored in a dark room with a temperature of 28° C. For the last 8 days they are stored at room temperature. From these culture tubes the next passage is inoculated. From the last passage (3rd one) the experimental plates and tubes are inoculated. The standardized medium for storage and the various stages is oatmeal agar, from which the actual experimental plates or, as the case may be, tubes are inoculated. In those cases, in which the formation of aerial mycelia on oatmeal agar is very weak or is entirely absent, glycerol-glycine agar is suggested for the standardized medium. Freshly prepared culture medium should always be used for each inoculation.

Petri dishes having a diameter of 9 cm with 15 cc culture medium should be used. Each plate will be inoculated with a fresh platinum-wire loop of spores.

Experimental Strains:
From his collection, Prof. Baldacci (Milano) is selecting 25 strains which, is possible, should represent all of his 21 series. The strains will be sent simultaneously (beginning of December, 1958) by him to all participants of the experiment. The strains are numbered consecutively from 1 to 25. Should some participants be able to work with only a few of the strains, only the first strains should be used, e.g. strains 1 to 10, or 1 to 15.

Culture Media:

1. Oatmeal agar: 20 g of oatmeal will be cooked in 1000 cc of tap water for 20 min. and filtered through cheesecloth, the filtrate should then be filled up to 1000 cc; addition of 18 g agar. Adjust to pH 7.2 with NaOH.

2. Glycerol-glycine agar:
(n. Lindenbein)

Glycerol (Merck)	20.0 g
Glycine (Merck)	2.5 g
K_2HPO_4 (Merck)	1.0 g
NaCl (Merck)	2.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$FeSO_4 \cdot 7H_2O$	0.1 g
$CaCO_3$	0.2 g
Agar (Difco)	18.0 g
Aq. dest.	1000 cc
Adjust to pH 7.2 with NaOH.	

3. Inorganic Salts-Starch Agar:
(n. Pridham, 1957)

Solution I: Difco soluble starch 10.0 g
Make a paste of the starch with a small amount of cold tap water and bring to a volume of 500 ml.

Solution II:	K_2HPO_4 (Merck)	1.0 g
	$MgSO_4 \cdot 7H_2O$	1.0 g
	NaCl	1.0 g
	$(NH_4)_2SO_4$	2.0 g
	$CaCO_3$	2.0 g
	Tap water	500 ml

Do not adjust pH. Mix the starch suspension and the salts solution. Add agar (Difco) 20.0 g.

4. Glycerol-asparagine agar:

Glycerol (Merck)	10.0 g
Asparagine (Merck)	1.0 g
K_2HPO_4 (Merck)	1.0 g
Agar (Difco)	20.0 g
Tap water	1000 cc
Adjust to pH 7.0 with NaOH.	

5. Peptone agar:

Bacto-Tryptone (Difco)	1.0 g
Bacto-Yeast Extract (Difco)	1.0 g
NaCl	8.5 g
Agar	17.0 g
Tap water	1000 cc

Sterilization of all media: 20 minutes at 120° C. pH is adjusted before sterilization.

Examination and Judgment of the Properties

1. Morphology of the aerial mycelium and the spores:

For the observation of the aerial mycelium, 5 Petri dishes with Starch-Ammoniumsulphate-Agar will be used for strain and repetition. The observation should be made on ripe aerial mycelia with a good spore formation. To establish the most favorable data for observation, a direct observation with a light microscope will be made on the 10th, 15th, and 20th days of growth; each time one plate will be used which will be discarded after the observation.

The form of the sporophores will be designated according to the nomenclature from Pridham (modified according to Baldacci):

Rectus	straight
Rectus-flexibilis	flexuous
Retinaculum-Apertum	open loops
Spira	spirals
Monoverticillus	
Monoverticillus-Spira	
Biverticillus	
Biverticillus-Spira	

The forms observed should, if possible, be designated. Photographs are also desired of typical and characteristic forms and of doubtful cases.

If an electron microscope is available, it is desirable to make print preparations from the same plate which is observed under the light microscope.

The results should be judged according to the designations:

smooth
spinous
hairy

2. Color of the aerial and substratum mycelia:

For judging the color of the aerial and substratum mycelia, 3 Petri dishes with glycerol-asparagine agar are used for each strain and repetition. The judging should be made after 10, 15 and 20 days. In those cases, in which an aerial mycelium is not formed, the upper side of the substratum mycelium is judged. In every case, however, the color of the reverse side will be designated as the color of the substratum mycelium. The judgment of color is carried out according to the 10 basic colors: colorless, white, yellow, orange, red, blue, green, gray, brown, and black. Intermediate tones are designated by putting the predominant color second and by underlining it on the file card. In the designation of the color, the age of the culture should be given in which the color was observed or, as the case may be, in which a change of color did not appear.

3. Formation of a melanoid pigment:

For the judging the formation of a melanoid pigment, 3 tubes with peptone agar (agar slant) will be used for each strain and repetition. The strains are inoculated in lines. The determination should be made after 2 and 4 days with

+ or - in comparison to an uninoculated control tube. Only the appearance of a brown-black coloring of the agar will be noted. The strength of the brown coloring need not be given.

Evaluation of the Experiment

Dr. Wallhäusser (Frankfurt/M. - Germany) will send each participant in the experiment 1 file card for each strain.* On this card, which has English notation, the entries of the properties observed are to be made separately for each repetition. On the back of the file card there is a space for special remarks as e.g. no growth on the precribed culture medium, use of a substitute culture medium, peculiarities in the morphology. After termination of the experiment, the completed cards will be returned to Wallhäusser for statistical evaluation. The concluding report will be compiled by Küster.

Schedule:

Beginning of Jan., 1959:	Carrying out of the 1st test.
Beginning of March, 1959:	Carrying out of the 2nd test.
Beginning of May, 1959:	Carrying out of the 3rd test.
Until July 1, 1959:	Return of the file cards to Dr. Wallhäusser.
October 1, 1959:	Distribution of the condensation of the result and the experimental report.

*See p. 65 This BULLETIN.

List of Participants

1.	Arishima, Dr.	Labor. in Meiji Seika Co., Kawasaki, Kanagawa-ken, Japan (25 strains)
2.	Backus, Dr. E.J.	Lederle Labor. Amer. Cyanamid Co., Pearl River, N.Y., U.S.A. (25 strains)
3.	Baldacci, Prof. Dr. E.	Ist. Patol. Vegetale, Univ. Milano, Via Celoria 2, Milano, Italy (25 strains)
4.	Ciferri, Prof. Dr. R.	Ist. Botan. Univ. Pavia, P. O. Box 165, Pavia, Italy (25 strains)
5.	Frommer, Dr. W.	Farbenfabrik Bayer, Wuppertal-Elderfeld, Germany (25 strains)
6.	Gause, Prof. Dr. G.F.	Inst. of Antibiotics, Acad. Med. Sci., Bolshaja Pirogovskaja, Moscow, U.S.S.R. (25 strains)
7.	Harada, Dr.	Labor. Kyowa Fermentation Co., Mishima, Shizuoka-ken, Japan (25 strains)
8.	Hata, Dr.	Kitasoto Inst., Minato-ku, Tokyo, Japan (25 strains)
9.	Hütter, Dr. R.	Inst. Spez. Botanik Eidg. Tech. Hochsch, Universitätsstr. 2, Zurich, Switzerland (25 strains)
10.	Joly, Dr. S.	Inst. Zimotecnio, Univ. de São Paulo, Piracicaba/São Paulo, Brazil (25 strains)
11.	Knöll, Prof. Dr.	Inst. Mikrobiol. u. experimentelle Therapie, Jena, Germany (25 strains)
12.	Komatu, Dr.	Inst. for Infect. Diseases, Tokyo Univ., Minato-ku, Tokyo, Japan (25 strains)
13.	Krassilnikov, Prof. Dr. N.A.	Inst. of Microbiol., Acad. Sci. U.S.S.R., Leninskij Prospekt 33, Moscow, U.S.S.R. (25 strains)
14.	Kurylowicz, Prof. W.	Inst. Antibiotikaforsch., Zurawai 6/12, Warazawa, Poland (25 strains)
15.	Mach, Dr. F.	Botan. Inst. Univ., Grimmer Strasse 88, Greifswald, Germany 25 strains)

16.	Nakazawa, Dr. K.	Inst. of Fermentation of Osaka Takeda Pharm. Co., Higaski Yodogawa-ku, Osaka, Japan (25 strains)
17.	Nishimura, Dr.	Labor. in Shionogi Pharm. Co., Dosho-machi, Osaka, Japan (25 strains)
18.	Nomi, Dr.	Inst. of Fermentation Technology, Hiroshima University, Hiroshima, Japan (25 strains)
19.	Ohara, Dr.	Yamanashi Univ., Yamanashi, Japan (25 strains)
20.	Okami, Dr. Y.	National Inst. Health, Minato-ku, Tokyo, Japan (25 strains)
21.	Otuki, Dr.	Ochanamizu Woman's Univ., Otuka, Tokyo, Japan (10 strains)
22.	Pfennig, Dr. N.	Inst. Mikrobiol. Univ., Gossler-strasse 16, Göttingen, Germany (10 strains)
23.	Pinnert, Dr. S.	Ste. Rhone-Poulenc, 9, Quai Jules-Guesde, Vitry-sur-Seine, France (25 strains)
24.	Pridham, Dr. T.G.	Northern Utiliz. Res. Div., Agric. Res. Serv., Peoria, Ill. U.S.A. (25 strains)
25.	Sakai, Dr.	Labor. in Fujisawa Pharm. Co., Honcho, Nihonbashi, Tokyo, Japan (25 strains)
26.	Sanchez-Marroquin, Dr. A.	Miami 40, Mexico 18/D.F., Mexico (25 strains)
27.	Shiobu, Dr.	Osaka Univ. Liberal Art Education (Hirano Branch) Higashi Sumiyoshi-ku, Osaka, Japan (25 strains)
28.	Szabo, Dr. J.	Inst. f. Bodenbiologie, Ungar. Akad. Wiss., Sopron, Hungary (10 strains)
29.	Tešić, Prof. Dr. Z.	Inst. Microbiol. du Sol, Univ. Beograd, Kojacka 1, Beograd, Yugoslavia (25 strains)
30.	Tomosugi, Dr.	Pfizer Co., Taito, Japan (25 strains)
31.	Umezawa, Dr.	Inst. Appl. Microbiol., Tokyo, Univ., Tokyo, Japan (25 strains)

32.	De Vries, Dr. G.A.	Centraalbureau voor Schimmelcultures, Baarn, Netherlands (10 strains)
33.	Waksman, Prof. S.A.	Inst. Microbiol., Rutgers Univ., New Brunswick, N.J., U.S.A. (25 strains)
34.	Wallhäusser, Dr. K.H.	Mikrobiol. Labor., Farbwerke Hoechst AG, Frankfurt/M., Hoechst, Germany (25 strains)

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DESIGNATION OF NEOTYPE CULTURES OF THE TYPE SPECIES OF CERTAIN GENERA OF THE FAMILY ENTEROBACTERIACEAE: SALMONELLA, SHIGELLA, ARIZONA, ESCHERICHIA, CITROBACTER AND PROTEUS*

Preliminary Statement

Editorial Board

A Commission was appointed by the Enterobacteriaceae Subcommittee to select strains to serve as neotype cultures of the type species of several of the genera of the family Enterobacteriaceae. The Commission reported a recommendation that neotype strains of the type species of six genera of the family be recognized, namely, Salmonella, Shigella, Arizona, Escherichia, Citrobacter and Proteus. A neotype strain was also recommended for an additional species of the genus Salmonella. The selections were made initially from strains in the National Collection of Type Cultures in London. The list of the names of the species with the catalogue number of the National Collection of Type Cultures was published as Appendix 3 of the Minutes of the International Enterobacteriaceae Subcommittee held at Stockholm in August, 1958 (The BULLETIN 8:179. 1958). Cultures of these recommended neotype strains have been deposited in the National Collection of Type Cultures in London and the American Type Culture Collection in Washington, D.C. They are available to other national collections that may desire them.

The Commission expects to recommend additional type and neotype strains of species of the Enterobacteriaceae.

Rule 9d Notes (b) and (c) of the International Code of Nomenclature of the Bacteria and Viruses defines "type culture" and "neotype cultures" as follows:

> (b) A type culture is a living culture of an organism which is a descendant of the original culture or isolation from which the author who first described the organism made his original description, which culture has been maintained pure, and which agrees in its characters with the original description.

(c) A neotype culture is one which has been accepted by international agreement to replace a type culture which is no longer in existence, it should agree with the diagnosis given by the original describer and should be recommended by those workers familiar with the species and their agreed recommendation approved by the Judicial Commission.

Species whose Neotype Strains have been Recommended

Names of Species	Catalogue No. National Collection of Type Cultures	Catalogue No. American Type Culture Collection
Salmonella cholerae-suis (Smith) Weldin 1927 Type species of genus Salmonella Lignieres 1900	5735	13312
Salmonella typhi-murium (Loeffler) Castellani and Chalmers 1919	74	13311
Shigella dysenteriae (Shiga) Castellani and Chalmers 1919 Type species of genus Shigella Castellani and Chalmers 1919	4837	13313
Arizona arizonae (Kauffmann) ? Type species of genus Arizona ?	8297	13314
Escherichia coli (Migula) Castellani and Chalmers 1919 Type species of genus Escherichia Castellani and Chalmers 1919	9001	11775
Citrobacter freundii (Braak) Werkman and Gillen 1932 Type species of genus Citrobacter Werkman and Gillen 1932	9750	8090

Proteus vulgaris Hauser 1885 Type species of genus Proteus Hauser 1885	4175	13315

The appended Table 1 gives the principal physiological characters of the neotype strains recommended. Other characters are those common to the members of the family Enterobacteriaceae.

As required by the Bacteriological Code of Nomenclature, the recommendations here recorded have been submitted to the members of the Judicial Commission for final action.

Table 1. Characters of proposed neotype strains.

Characters	Salmonella cholerae-suis NCTC 5735 ATCC 13312	Salmonella typhi-murium NCTC 74 ATCC 13311	Shigella dysenteriae NCTC 4837 ATCC 13313	Arizona arizonae NCTC 8297 ATCC 13314	Escherichia coli NCTC 9001 ATCC 11775	Citrobacter freundii NCTC 9750 ATCC 8090	Proteus vulgaris NCTC 4175 ATCC 13315
Motility	+	+	-	+	+	+	+
Fermentations:							
Glucose (acid)	+	+	+	+	+	+	+
Glucose (gas)	+	+	-	+	+	+	+
Arabinose	-	+	-	+	+	+	-
Xylose	+	+	-	+	-	+	+
Rhamnose	+	+	-	+	+	+	-
Lactose	-	-	-	x	+	+	-
Sucrose	-	-	-	-	-	+	+
Maltose	+	+	x	+	+	+	+
Trehalose	-	+	+	+	+	+	x
Glycerol	(+)	(+)	-	+	+	+	(+)
Mannitol	+	+	-	+	+	+	-
Dulcitol	(x)	+	-	-	-	-	-
Sorbitol	+	+	-	+	+	+	-
Inositol	-	+	-	-	-	(x)	-
Adonitol	-	-	-	-	-	-	-
Salicin	-	-	-	-	x	(x)	-
Sorbose	-	-	-	-	+	+	-
Stern's Glycerol	-	+	-	+	-	+	-
Gelatin hydrolysis	-	-	-	x	-	-	+
Indole	-	-	-	-	+	-	+
H_2S	-	+	-	+	-	+	+
Ammonium citrate	+	+	-	+	-	+	-

Sorbose	-	-	-	-	+	+	
Stern's Glycerol	-	+	-	+	-	+	-
Gelatin hydrolysis	-	-	-	x	-	-	+
Indole	-	-	-	-	+	-	+
H_2S	-	+	-	+	-	+	+
Ammonium citrate	+	+	-	+	-	+	-
Koser's citrate	-	+	-	(+)	-	+	-
Christensen's citrate	+	+	-	+	-	+	-
Methyl Red	+	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-	-
Urease	-	-	-	-	-	-	+
Nitrate reduction	+	+	+	+	+	+	+
Catalase	+	+	-	+	+	+	+
Phenylalanine	-	-	-	-	-	-	+
Gluconate	-	-	-	-	-	-	-
Malonate	-	-	-	+	-	-	-
Potassium cyanide	-	-	-	-	-	+	+
Decarboxylases*							
Lysine	+	+	-	+	+	-	-
Arginine	+	+	+	+	+	+	-
Ornithine	+	+	-	+	+	-	-
Glutamic acid	-	-	+	-	+	-	-
Organic acids (salts of):**							
d-tartrate	+	+	-	-	-	+	-
citrate	+	+	-	x	-	+	-
mucate	-	+	-	+	-	+	-
l-tartrate	-	+	-	-	-	-	-
i-tartrate	+	+	-	-	-	-	-

+ = positive 1-3 days
x = delayed reaction
(+) = variable (different workers got different results)
(x) = variable and delayed (different workers got different results)

*Results obtained by Dr. V. Møller
**Results obtained by Dr. F. Kauffmann